CHARACTERIZATION OF THE ONTOGENY AND INTER-INDIVIDUAL VARIATION OF GENES IN CHOLESTEROL AND STATIN PATHWAYS

By

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Abstract

Introduction: Previous studies have shown that gene expression can change throughout development,[1] and therefore genotype-phenotype associations found in adults might not be observed in children of all ages. The purpose of this study was to 1) characterize the ontogeny of 30 genes in pathways related to cholesterol synthesis and/or statin action or toxicity in pediatric liver samples, and 2) assess the *in vitro* and *in vivo* consequences in children of genetic variation in 3-hydroxy-3-methyl-glutaryl-Coenzyme A reductase (*HMGCR*) and heterogeneous nuclear ribonucleoprotein A1 (*HNRNPA1*), two genes implicated in altered cholesterol levels and/or statin response.[2,3]

Methods: RNA and DNA were isolated from pediatric liver samples (n=62), and DNA was isolated from patients in the Cardiology Pharmacogenomic Repository (CPR) (n=195). For Aim 1, the ontogeny of mRNA expression from 30 genes related to cholesterol and statin metabolism as measured by RNA-seq in the liver samples was assessed. For Aim 2, all postnatal samples were genotyped for rs1920045 (*HNRNPA1*) and rs3846662 (*HMGCR*), and genotypes were tested for association with either alternative splicing *in vitro* (liver samples) or plasma lipid levels *in vivo* (CPR). Statistical analyses on liver sample data were conducted with Kruskal-Wallis or Wilcoxon tests with Bonferroni correction. Analysis of CPR samples was completed with ANOVA, Kruskal-Wallis or Wilcoxon tests with Bonferroni correction. Samples were stratified by race and analyses were repeated. Liver sample use was deemed non-human subjects research and the CPR was approved by the Pediatric IRB.

Results: Analysis of postnatal liver samples revealed age-related changes in Ensembl-based total and primary mRNA transcript expression of *ABCB1* ($p<0.0008$). Inclusion of prenatal samples revealed 13 additional genes with age-related expression changes during development in either

the Ensembl or UCSC based data. The ratios of alternative to canonical transcripts of *HMGCR* trended towards significance in the *HMGCR* and *HNRNPA1* dominant genotype models (p=0.0465, 0.0470 respectively). CPR analysis suggested a relationship between *HMGCR* genotype and low-density lipoprotein cholesterol (LDL-C) or total cholesterol (TC) in African Americans, or *HNRNPA1* genotype and TC in Caucasians, but these relationships did not achieve statistical significance.

Conclusion: Although trends in age-related changes in gene expression, and genotype-phenotype associations, were observed for several genes of interest, the number of statistically significant associations was limited by use of a stringent criterion for multiple testing as well as intra-group variability and relatively small sample sizes given the amount of variability observed. Until these issues are resolved in a larger number of samples, it is premature to conclude genotype-phenotype associations observed in adults will also be present in children at all ages.

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INTRODUCTION

Between childhood and early adulthood, significant precursors to cardiovascular disease (CVD), begin forming in coronary arteries.[4] The Bogalusa Heart Study found that the presence of fatty streaks in coronary arteries increased in prevalence from 50% at 2 – 15 years of age to 85% at 21 – 39 years of age, and the presence of raised fibrous plaques increased in prevalence from 8% to 69% during this time.[4] Additionally, the emerging childhood obesity epidemic has accelerated the concern over CVD risk factor development among children.[5]

In response, the National Heart, Lung, and Blood Institute (NHLBI) released updated pediatric cardiovascular health guidelines in 2011, which recommended universal lipid screening for children between nine and 11 years of age.[6] Lipid lowering therapy during adolescence is one focus of the updated guidelines, and statins are recommended as a first-line pharmacotherapeutic option for children with elevated lipid levels.[6] Statins constitute a medication class that acts primarily by inhibiting 3-hydroxy-3-methylglutaryl-Coenzyme A reductase (HMGCR) in the liver, which is the rate-limiting enzyme in endogenous cholesterol synthesis. Recently, it was estimated that over 200,000 children qualify for statin use in the U.S.[7]

There are presently seven FDA-approved drugs within the statin class: atorvastatin, fluvastatin, lovastatin, pitavastatin, pravastatin, rosuvastatin, and simvastatin.[8] Transporters are involved in the import of some statins into hepatocytes.[9] Once in the hepatocyte, statins exert their clinical effect on the cholesterol pathway, can be transformed by multiple enzymes into active or inactive forms, and are later excreted into the bile by transporter proteins.[10,11] Due to the well-defined pathways involved in their pharmokinetics and pharmacodynamics, statins offer a unique and important opportunity to explore genetic ontogeny and variation involved with their disposition and molecular effect as it relates to clinical response.

Within the medical community, there exists some concern over disrupting cholesterol metabolism during childhood development.[12] Lipids and sterols are involved in neuronal development as well as synthesis of steroid hormones, key components of development.[12] Statins have been shown to be safe and efficacious in pediatric populations; however, much of this data comes from studies with relatively short follow-up periods.[13] Considering how statins are commonly used long-term, which could equate to decades if initiated in childhood, the safety of statin use during childhood and adolescence has yet to be fully determined.[13] Initial studies have shown conflicting results surrounding the impact of statins on hormone levels, and neuronal effects, if any, may not be apparent until much later on in life.[12,13] A study to fully address these concerns would likely be resource and time intensive. Surrogate endpoints, such as lipid levels and liver enzymes, are currently used to assess both the efficacy and safety of statins in children.[12] Additional information could be gained from characterizing the genetic background of statin therapeutic and metabolic pathways during development.

It is widely recognized that gene expression changes as children grow and develop;[1] however, the molecular-level details of this process for many genes involved in statin pharmacokinetics and pharmacodynamics are largely unknown. Studies on some of the most common drug metabolizing enzymes, cytochrome P450 (CYP) enzymes, have shown that the expression of these genes changes throughout childhood development.[14] This suggests that the expression ontogeny of other drug metabolizing and transporting genes could also be dynamic and thus greatly affecting pharmacokinetics and pharmacodynamics in pediatric patients. Although the ontogeny of CYP enzymes has been extensively characterized, targets of statin action remain ontogenetically undescribed.

Additionally, multiple genes have been identified that may contribute to variability in statin response.[15] For instance, some statins or statin metabolites have been shown to be substrates of a drug transporter, MDR1, encoded by the gene *ABCB1*,[16] and variation within this gene has shown some association with response to treatment with particular statins;[17,18] however, the effect may not be clinically significant.[17] Our research team and a collaboration in the Netherlands have published on its hepatic ontogeny in prenatal, neonatal and a small group of pediatrics, yet *ABCB1*'s hepatic expression at different points in childhood was not fully investigated.[19] An additional gene, *SLCO1B1*, codes for a transporter thought to import statins into the hepatocyte.[20] In adults, genetic variation in this gene has been associated with increased plasma statin levels and clinical myopathy, yet in children it was shown to be associated with decreased plasma drug levels.[21,22] Furthermore, Gryn and Hegel published a review describing genetic variation in dozens of genes, such as *HMGCR* and *COQ2*, which have been associated with statin therapy in adults[15] and has yet to be fully investigated in the pediatric setting.

Recently, an alternative transcript of the statin target, HMGCR, that lacks exon 13 (HMGCRΔ13) has gained attention for its association with reduced response to statins in adults.[3] A variant in *HMGCR*, rs3846662, was associated with increased expression of HMGCRΔ13 relative to the expression of the canonical HMGCR transcript including exon 13 (HMGCRex13) in Caucasian lymphoblastoid cell lines (LCLs).[23] *HMGCR* has a binding motif for heterogeneous nuclear ribonucleoprotein A1 (HNRNPA1) in the intronic region between exons 13 and 14 (**Figure 1**).[2] *HNRNPA1* has been shown to impact the alternative expression of *HMGCR*; however the relationship was not investigated in the dynamic metabolisms of children.[2] Additionally, it was shown that a variant upstream of *HNRNPA1*, rs1920045, was associated with altered expression of an alternative transcript of *HNRNPA1* that included exon-8

(HNRNPA1ex8) versus the canonical transcript that excluded exon 8 (HNRNPA1⊿8) (Yu, *et al.*, Supplemental Figure S6-A).[2] This splice variant, HNRNPA1ex8, in turn was hypothesized to increase expression of HMGCR⊿13.[2] It is therefore hypothesized that 'T/T' genotype will alter relative HMGCR⊿13 expression. The transcript of HMGCR⊿13 may have clinical significance, as a relative increase in expression of the transcript has been associated with cholesterol-lowering in response to statins, and SNP rs3846662 linked to this expression is associated with lipid levels in adults.[3,23,24]

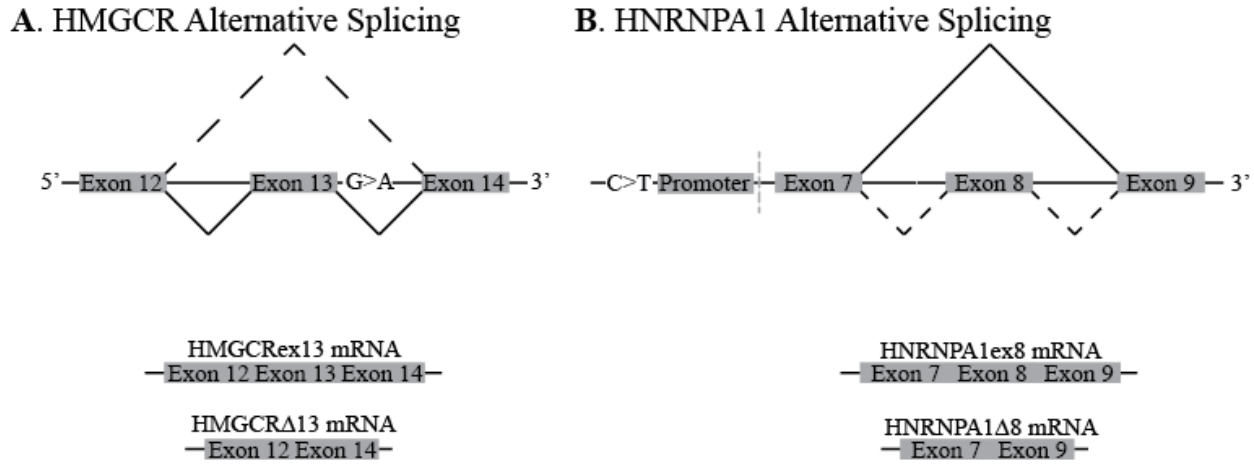

Figure 1. Canonical and Alternative Splicing of *HMGCR* (**A**) and *HNRNPA1*(**B**). Solid lines indicate the splicing pattern for the canonical transcript. Dashed lines indicate the splicing pattern for the alternative transcript.[2]

Phenotypic variation due to genetic differences that have been observed in adults may only be apparent in children once gene pathways are fully developed. In order to determine this, the developmental trajectory of gene expression must be described. Additionally, genetic variants with known phenotypes in adults need to be studied in children to discern whether the phenotype-genotype associations remain consistent. The objectives of this study were to (1) characterize the ontogeny of mRNA expression in pediatric liver samples for a set of 30 genes in pathways associated with cholesterol synthesis and/or statin therapeutic response or toxicity, and

(2) investigate the consequences of genetic variation in *HMGCR* and *HNRNPA1* in a pediatric context, both *in vitro* and *in vivo*.

METHODS

Tissue Repository

Human liver tissues were obtained from two National Institute of Child Health and Development supported tissue repositories – the University of Maryland Brain and Tissue Bank for Developmental Disorders (Baltimore, MD) and the Laboratory of Human Development at the University of Washington (Seattle, WA), the Liver Tissue Cell Distribution System, and Xenotech, LLC (Lenexa, KS). The age of removal of liver samples ranged from prenatal (103 days post-conception) to late adolescence (17 years). All individuals were deceased at the time of removal and the causes of death varied. The use of this repository has been deemed non-human subjects research by the Children's Mercy Hospital (CMH) Pediatric Institutional Review Board (IRB). Characteristics of the samples used from the liver repository are described in **Table 1**. RNA and DNA were extracted from liver tissues with the Qiagen AllPrep DNA/RNA Mini Kit or the Qiagen AllPrep DNA/RNA/miRNA Universal Kit (Hilden, Germany) and stored at -80^{o}C and 4^{o}C, respectively.

RNA samples for RNA-sequencing (RNA-seq) were selected based on the absence of documented liver disease or medications affecting the liver, representative distribution across developmental ages, and an RNA Quality Index (RQI) above 3. RQI is a quality measure, analogous to RNA Integrity Number (RIN) fom the Agilent Bioanalyzer, on a scale from zero to 10, with 10 being considered the highest quality. The RNA was run in a microfluidic StdSens chip on an Experion^{TM} (Bio-Rad, Hercules, CA).

Table 1. Characteristics of Liver Tissue Samples (n=61).			
Characteristic			**N**
Age at Time of Removal			
	Group 0	(prenatal)	10
	Group 1	(birth to <1 year)	14
	Group 2	(1 to ≤6 years)	16
	Group 3	(>6 to ≤12 years)	13
	Group 4	(>12 years)	8
Sex			
	Male		45
	Female		16
Race			
	Caucasian		18
	African American		8
	Other, Multiple, Unknown		35

Living Repository – Cardiology Pharmacogenomics Repository (CPR)

The Cardiology Pharmacogenomics Repository (CPR) is a living patient repository with DNA isolated from biospecimens (i.e. saliva, blood) of each participant, using the Sigma GeneElute™ Mammalian Genomic DNA Miniprep Kit (St. Louis, MO) or QIAamp® DNA Blood Mini Kit (250) (Hilden, Germany) according to manufacturer protocols. The enrollment criteria include: age 0 to 26 years, patients with existing cardiac disease, patients at risk of CVD (i.e. abnormal lipid profile, obesity, diabetes mellitus, family history of cardiac disease), and/or patients receiving a cardiovascular pharmacotherapeutic. Participants were recruited from the nutrition, weight management, general cardiology, and preventative cardiology clinics at CMH. The samples were coded to protect patient privacy but could be linked back to participants' Electronic Medical Records (EMR) by the Principal Investigator for retrospective chart review. All samples in the CPR as of February 25, 2015 (n = 195) were included in this study, except for one participant who had missing data on all measurements. A total of eight patients were

subsequently excluded: five patients who had been enrolled following cardiac transplantation and three who had no lipid panel documented in their EMRs prior to statin therapy (final n=186). The CPR was approved by the CMH Pediatric IRB.

CPR Clinical Data Collection

Retrospective chart review was conducted to collect values for total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides, height, weight, body mass index (BMI), age (in years and months) at time of lab draw, and sex for each CPR participant. Information regarding documented lipid-lowering medication use at the time of lab draw was also collected. Lipid values were taken from the most recently documented lab draw up to and including the intervention visit, except one participant, whose lipid values after the visit were used. The BMI was taken from the intervention visit documentation. To get the correct age from the data for BMI percentile calculation, the month difference between the lipid panel date and the visit date was calculated and then added to the age at time of the lab. There were two participants for which this calculation could not be completed. This calculated age was then used for calculation of the BMI percentile. Race information was taken from documentation of the participant's self-reported race at the time of his or her enrollment. Races were inclusive of those identifying with a Hispanic ethnicity for analysis (i.e. Caucasian race with non-Hispanic ethnicity was included with Caucasian race with Hispanic ethnicity, and the same was done for ethnically Hispanic African Americans). Characteristics of the CPR participants are described in **Table 2.** The collection of this information was performed with the CPR Principal Investigator, Dr. Jon Wagner, to ensure the most appropriate dates were selected and the correct information was recorded.

Table 2. Characteristics of CPR Participants (n=186).	
Characteristic	**N**
Sex	
Male	92
Female	94
Race	
Caucasian	138
Hispanic Ethnicity	30
Non-Hispanic Ethnicity	108
African American	27
Hispanic Ethnicity	1
Non-Hispanic Ethnicity	26
Other, Multiple, Unknown	21
BMI Category	
Underweight	3
Normal Weight	29
Overweight	26
Obese	126
Unknown	2
Lipid Lowering Therapy	
Fish Oil	15
Red Yeast Rice	1
Clinical Characteristics (NHLBI "Acceptable Concentration")[6]	**Mean (SD)**
Age, years	12 (3.2)
Baseline Total Cholesterol, mg/dL (<170 mg/dL)	242 (52)
Baseline LDL Cholesterol, mg/dL (<110 mg/dL)	166 (52)
Baseline HDL Cholesterol, mg/dL (>45 mg/dL)	47 (13)
Baseline Triglycerides, mg/dL (Age 0 to 9 yrs, <75 mg/dL Age 10 to 19 yrs <90 mg/dL)	151 (81)

RNA-seq and Bioinformatics

RNA-seq was completed for 62 liver tissue samples from the repository. One µg total human liver RNA was used to generate TrueSeq Ribo-Zero libraries with the Illumina TruSeq Stranded Total RNA with Ribo-Zero Globin Library Prep Kit. Samples were run on the Illumina

HiSeq 1500 (Genomics Research Core Lab) with paired-end (2 x101), deep sequencing coverage (104x).

RNA-seq reads were output in FASTQ format, and the data were organized by individual samples. Quality control was performed with FastQC. No sequence trimming or removal was performed. Two reference transcriptomes were prepared, one from the European Molecular Biology Laboratory (Ensembl) database and one from the University of California Santa Cruz (UCSC) genome database. RNA-seq Expectation Maximization (RSEM) was performed with Bowtie2 to index the reference transcriptomes and align the sample reads. Based on the maximum likelihood model, RSEM was run to assign reads to annotated transcripts and estimate the abundance of each transcript. In data analysis, one sample was removed as a library size outlier.

Genes of interest for this study were identified from statin pharmacodynamic and pharmacokinetic pathways (PharmGKB),[10,11] reviews of the pharmacogenomics of statin disposition and response,[15,25,26] and genome-wide association studies of variability in cholesterol levels (for example Tikkanen, *et al*. and citations therein).[27] The query included total and primary transcripts of *ABCB1*, *AGTR1*, *AMPD1*, *APOC1*, *APOC2*, *APOE*, *ATP2B1*, *CETP*, *CLMN*, *CPT2*, *COQ2*, *CYP7A1*, *DMPK*, *DNAJC5B*, *GATM*, *HMGCR*, *HNRNPA1*, *HTR3B*, *HTR7*, *KIF6*, *LDLR*, *LPA*, *LPIN1*, *MYLIP*, *NOS3*, *PCSK9*, *PYGM*, *RYR1*, *SLC10A1*, and *SLCO1B1*. This set of genes was analyzed for total and primary transcript mRNA expression changes associated with age as a categorical variable, from prenatal (103 days post-conception) to age 17 years.

Additionally, the RNA-seq dataset was queried for mRNA expression changes associated with *HMGCR* rs3846662G>A and *HNRNPA1* rs1920045C>T. The transcripts of interest were *HMGCR* transcripts including and excluding exon 13 (ENST00000287936 and

ENST00000343975, respectively) and *HNRNPA1* transcripts including and excluding exon 8 (ENST00000340913 and ENST00000547276, respectively). This SNP-associated gene expression had been previously described in LCLs.[2,23]

Genotyping

All postnatal DNA samples (liver and CPR) in this study were genotyped for *HMGCR* rs3846662G>A and *HNRNPA1* rs1920045C>T using TaqMan® SNP genotyping assays C__2838669_10 and C__12057681_10, respectively (LifeTechnologies, Carlsbad, CA), with KAPA Probe Fast qPCR Master Mix (2x) ABI Prism® (KAPA Biosystems, Boston, MA) on an AB 7900HT Fast Real-Time PCR System (Applied Biosystems). For each reaction, 3 to 19 ng of DNA were used, in a total volume of 6 uL, with the exception of one sample that amplified with less than 0.53 ng of DNA in this volume. If amplification of a sample failed, the input DNA was doubled. The cycling conditions for both assays were 95°C, 2 min, [95°C, 10 sec, 60°C, 60 sec] for 45 cycles. DNA samples from the Coriell Institute for Medical Research were used as controls (*HMGCR*, G/G: NA17294, *HMGCR*, G/A: NA12813, *HMGCR*, A/A: NA12877; *HNRNPA1*, C/C: NA12882, *HNRNPA1*, C/T: NA17204, *HNRNPA1*, T/T: NA06989). Ten percent of samples were randomly selected and run a second time to check for consistency. For each marker, call rates were computed (100%) along with Hardy-Weinberg Equilibrium (HWE) tests by race (**Table 3)**.

Table 3. Genotype Information of Samples. AA: HMGCR G/G or HNRNPA1 C/C, AB: Heterozygous, BB: HMGCR A/A or HNRNPA1 T/T

					Total Genotype, N				Cau. Genotype, N				Af. Am. Genotype, N			
Gene	**RsID**	**Cohort**	**Position**	**Alleles**	**AA**	**AB**	**BB**	**HWE p-value**	**AA**	**AB**	**BB**	**HWE p-value**	**AA**	**AB**	**BB**	**HWE p-value**
HMGCR	rs3846662	RNA-seq	Chr. 5	G>A	16	23	12	0.5091	5	8	5	0.6374	6	2	0	0.6862
		CPR			69	76	41	0.0254	38	62	38	0.2334	21	6	0	0.5160
HNRNPA1	rs1920045	RNA-seq	Chr. 12	C>T	21	22	8	0.5809	6	9	3	0.9035	1	4	3	0.8504
		CPR			50	87	49	0.3791	49	67	22	0.9096	0	8	19	0.3662

Statistical Analysis

Association of age with gene expression measured by RNA-seq for the 30 genes of interest was carried out using Kruskal-Wallis tests (i.e., non-parametric ANOVA) with Bonferroni correction (significance: $p<0.0016$). Age was treated as a categorical variable and gene expression - in transcripts per million reads (TPM) - was continuous. Age groups were based on developmental stages (Group 0: prenatal, Group 1: birth to <1 year, Group 2: 1 to ≤6 years, Group 3: >6 to ≤12 years, and Group 4: >12 years). Categorical age group analysis was selected over continuous age analysis to allow detection of non-linear expression changes that may occur during development. Gene expression was evaluated as total transcript expression and primary transcript expression. The most abundant transcript in the RNA-seq output was chosen as the primary transcript. The significance threshold with Bonferroni correction was higher for the primary transcript analysis because only 27 genes had more than one transcript from which to choose a primary transcript in the RNA-seq output for the Ensembl dataset and only 13 genes had more than one transcript in the UCSC dataset ($p<0.0018$ and $p<0.0038$, respectively). Separate analyses were performed including and excluding the prenatal samples. Pairwise comparisons were performed with Tukey's HSD or Steel-Dwass tests.

The ratios of alternative to canonical transcript expression for *HMGCR* and *HNRNPA1* were calculated from the measured RNA-seq transcript data and log-transformed. Associations of log-transformed ratios with the genotypes of interest were assessed using Wilcoxon (chi-square approximation to the one-way test) or Kruskal-Wallis tests with Bonferroni correction. Both general genetic (co-dominant) and dominant genetic models were performed, and ratios of gene expression were viewed as continuous variables. The samples were stratified by race and all

analyses were repeated for the Caucasian subgroup. There was an insufficient number of African American samples to perform separate analyses for this subgroup.

Associations between the genotypes (*HMGCR* or *HNRNPA1*) and lipid levels (TC, LDL-C, HDL-C, or triglycerides) were evaluated using ANOVA with log-transformed lipid levels and genotype modeled with a general genetic (co-dominant) model. BMI percentiles were calculated from the raw BMI, sex, and age at BMI visit. These percentiles were categorized into underweight ($<5^{th}$ percentile), normal (5^{th} to $<85^{th}$ percentile), overweight (85^{th} to $<95^{th}$ percentile), or obese ($\geq 95^{th}$ percentile) according to the guidelines from the Center for Disease Control (CDC). One participant was over 20 years old, and thus was classified according to the adult CDC guidelines. BMI was then analyzed as a categorical variable with a contingency table and Chi-square or Fisher's Exact test. Due to fewer than 3 participants being classified as underweight, this category was excluded from the BMI analysis. Samples were stratified by race and all CPR analyses were repeated for Caucasians and African Americans separately using Kruskal-Wallis or Wilcoxon tests with Bonferonni correction.

All statistical analysis was conducted in JMP®, Version 10. SAS Institute Inc., Cary, NC, 1989-2007 or R version 3.1.2 (www.R-project.com).[28]

RESULTS

Hepatic Ontogeny

The Ensembl-based total gene expression ontogeny analysis of the gene set including prenatal samples revealed 12 genes with expression changes significantly associated with age: *ABCB1* ($p<0.0001$), *APOC1* ($p=0.0008$), *ATP2B1* ($p<0.0001$), *CETP* ($p<0.0001$), *COQ2* ($p=0.0003$), *CYP7A1* ($p<0.0001$), *DMPK* ($p<0.0001$), *HMGCR* ($p=0.0002$), *HNRNPA1* ($p<0.0001$), *KIF6* ($p<0.0001$), *MYLIP* ($p<0.0001$), and *RYR1* ($p=0.0005$) (**Table 4**). In pairwise

comparisons for each of these genes, the expression in Group 0 (prenatal) differed from the expression in at least one other group (**Table B1**). Analysis of the transcripts aligned to the UCSC database showed significant age associations in the same genes as in the Ensembl analysis, except for *COQ2* (p=0.0059) (**Table 4**). Two additional genes were significant in the UCSC-based analysis: *APOC2* (p=0.0003) and *LPIN1* (p<0.0001). In pairwise comparisons for these two genes, the expression in Group 0 (prenatal) differed from the expression in at least one other group (**Table B1**).

The Ensembl-based primary transcript expression analysis including prenatal samples revealed significant associations between age and the expression of *ABCB1* (p<0.0001), *APOC1* (p=0.0008), *CETP* (p=0.0002), *CLMN* (p=0.0003), *COQ2* (p=0.0002), *DMPK* (p=0.0001), *HMGCR* (p=0.0001), *HNRNPA1* (p=0.0002), *KIF6* (p=0.0005), *LPIN1* (p=0.0002), and *MYLIP* (p<0.0001) (**Table 5**). Pairwise analysis showed that Group 0 (prenatal) gene expression differed from the expression in at least one other age group for each of these genes (**Table B2**). All of these genes that also had primary transcripts in the UCSC-based data showed an analogous relationship between age and primary UCSC transcript expression, with the exception of *CETP* (p=0.1644) (**Table 5**).

Due to the different cellular environment of the prenatal liver compared to the postnatal liver, separate analyses were performed excluding the prenatal samples. In the Ensembl-based total gene expression ontogeny analysis of the 30 genes of interest, excluding prenatal samples, only one gene's expression, *ABCB1,* showed significant association with age (p=0.0008) (**Table 4**). There was an increase in *ABCB1* expression during development, with Group 1 (<1 year-old) showing significantly different gene expression from other groups (**Figure 2; Table 6)**. In the UCSC alignment, *ABCB1* expression trended toward a significant relationship with age but did

not reach significance (p=0.0024) (**Figure 3**). No other gene's expression showed a significant relationship with age in the UCSC-based analysis (**Table 4**).

In the Ensembl-based primary transcript expression ontogeny analysis of the gene set excluding prenatal samples, only *ABCB1* (ENST00000265724) expression was significantly associated with age (p=0.0008) (**Table 5**). Group 1 (<1 year old) *ABCB1* ENST00000265724 expression differed from other age groups and expression appeared to increase with age (**Figure 4; Table 6**).

Table 4. Total Gene mRNA Expression Ontogeny Analysis Results.

	Kruskal-Wallis p-value Excluding Prenatal		Kruskal-Wallis p-value Including Prenatal	
Gene	**Ensembl**	**UCSC**	**Ensembl**	**UCSC**
ABCB1	0.0008	0.0024	<0.0001	<0.0001
AGTR1	0.4262	0.2991	0.0315	0.4054
AMPD1	0.4035	0.5261	0.2105	0.5888
APOC1	0.3978	0.2023	0.0008	<0.0001
APOC2	0.6872	0.4746	0.0480	0.0003
APOE	0.8336	0.6200	0.6856	0.1382
ATP2B1	0.4890	0.0246	<0.0001	<0.0001
CETP	0.6463	0.4240	<0.0001	0.0012
CLMN	0.9193	0.3689	0.0021	0.0019
COQ2	0.2007	0.0443	0.0003	0.0059
CPT2	0.4767	0.4561	0.1604	0.0121
CYP7A1	0.0041	0.0039	<0.0001	<0.0001
DMPK	0.0332	0.0072	<0.0001	<0.0001
DNAJC5B	0.6655	0.1696	0.6987	0.1126
GATM	0.7875	0.7614	0.5973	0.6912
HMGCR	0.9994	0.9632	0.0002	0.0002
HNRNPA1	0.9987	0.7840	<0.0001	0.0001
HTR3B	0.8304	0.5911	0.1170	0.3277
HTR7	0.0630	0.0377	0.0636	0.0713
KIF6	0.0043	0.0084	<0.0001	<0.0001
LDLR	0.5213	0.3911	0.5694	0.0973
LPA	0.8900	0.9933	0.2358	0.0655
LPIN1	0.1658	0.0100	0.0147	<0.0001
MYLIP	0.6914	0.3682	<0.0001	<0.0001
NOS3	0.9440	0.9991	0.1903	0.9723
PCSK9	0.2771	0.1198	0.0024	0.0279
PYGM	0.7973	0.9830	0.6099	0.9952
RYR1	0.0993	0.0494	0.0005	0.0005
SLC10A1	0.2400	0.1682	0.0280	0.0093
SLCO1B1	0.6535	0.3704	0.0585	0.0018

Table 5. Primary Transcript Expression Ontogeny Analysis Results.

	Kruskal-Wallis p-value Excluding Prenatal		Kruskal-Wallis p-value Including Prenatal	
Transcript	**Ensembl**	**UCSC**	**Ensembl**	**UCSC**
ABCB1 ENST00000265724	0.0008		<0.0001	
AGTR1 ENST00000402260 or NM_032049	0.6082	0.1031	0.5421	0.2008
AMPD1 ENST00000520113	0.6616		0.4338	
APOC1 ENST00000252491	0.3850		0.0008	
APOC2 ENST00000252490	0.7010		0.0727	
APOE ENST00000252486	0.8855		0.7680	
ATP2B1 ENST00000261173 or NM_001682	0.2835	0.3450	0.0037	0.2320
CETP ENST00000200676 or NM_000078	0.9837	0.9450	0.0002	0.1644
CLMN ENST00000556454	0.8287		0.0003	
COQ2 ENST00000311469	0.0174		0.0002	
CPT2 ENST00000371486	0.4943		0.1418	
DMPK ENST00000600757 or NM_001081563	0.0917	0.0134	0.0001	<0.0001
DNAJC5B ENST00000519330	0.4474		0.1457	
GATM ENST00000396659	0.6792		0.6852	
HMGCR ENST00000287936 or NM_000859	0.9842	0.8845	0.0001	0.0002
HNRNPA1 ENST00000547276 or NM_002136	0.9989	0.6184	0.0002	0.0001
HTR3B ENST00000260191	0.9430		0.0893	
HTR7 ENST00000277874 or NM_000872	0.3918	0.0473	0.3699	0.0741
KIF6 ENST00000373213 or NM_001289020	0.1453	0.0015	0.0005	<0.0001
LDLR ENST00000252444 or NM_001195799	0.6544	0.0973	0.3915	0.0257
LPA ENST00000316300	0.8790		0.2254	
LPIN1 ENST00000256720 or NM_145693	0.0121	0.0186	0.0002	<0.0001
MYLIP ENST00000349606	0.6356		<0.0001	
NOS3 ENST00000297494 or NM_000603	0.9306	0.7174	0.3521	0.6297
PCSK9 ENST00000302118	0.2274		0.0051	
PYGM ENST00000164139 or NM_001164716	0.6174	0.9177	0.7589	0.9767
RYR1 ENST00000600337 or NM_001042723	0.3779	0.0305	0.5212	0.0041

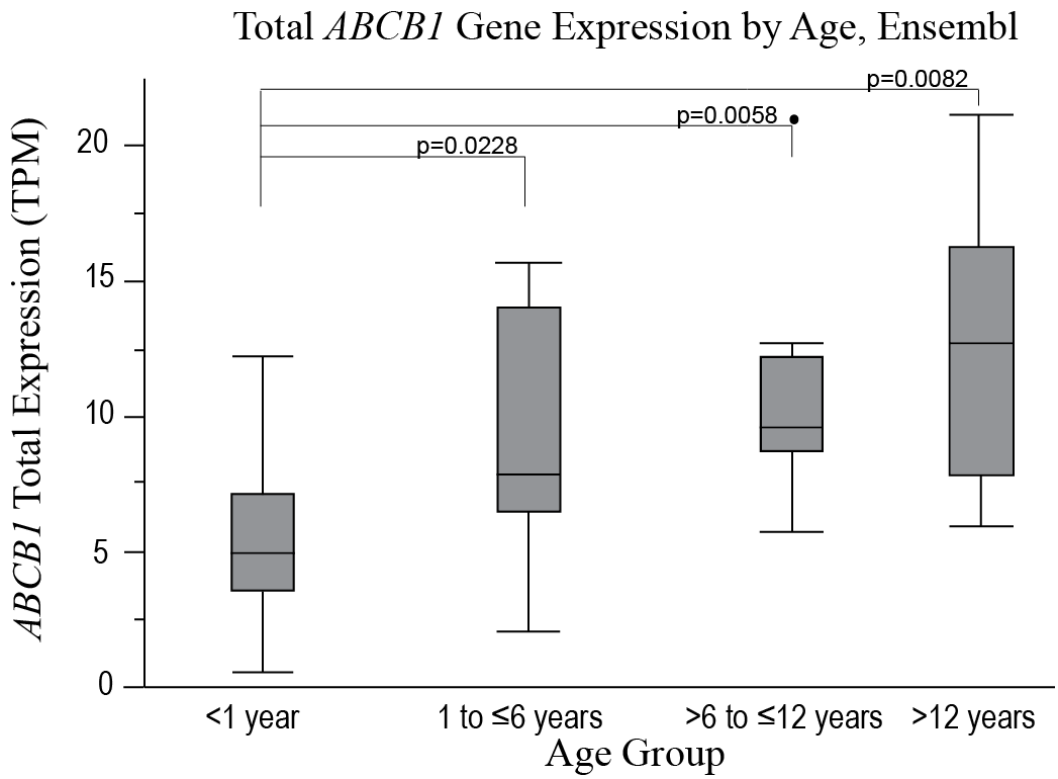

Figure 2. Total Expression (Ensembl-based) of *ABCB1* by Age Group. Pairwise comparison p-values presented above box-plots.

Table 6. Mean Expression by Age Group for Genes of Significance, Excluding Prenatal Samples. Zero imputed for negative values in lower limit of CI.

	Group	Mean (95% CI), TPM	Kruskal Wallis p-value
ABCB1 Total Expression	1	5.583 (3.86, 7.31)	0.0008
	2	9.475 (7.34, 11.61)	
	3	10.545 (8.35, 12.74)	
	4	12.650 (8.51, 16.79)	
ABCB1 ENST00000265724	1	3.551 (2.31, 4.79)	0.0008
	2	6.696 (4.93, 8.47)	
	3	7.847 (5.88, 9.81)	
	4	8.899 (5.86, 11.94)	
KIF6 NM_001289020	1	1.415 (0.50, 2.33)	0.0015
	2	0.008 (0, 0.02)	
	3	0.025 (0. 0.05)	
	4	0.036 (0, 0.12)	

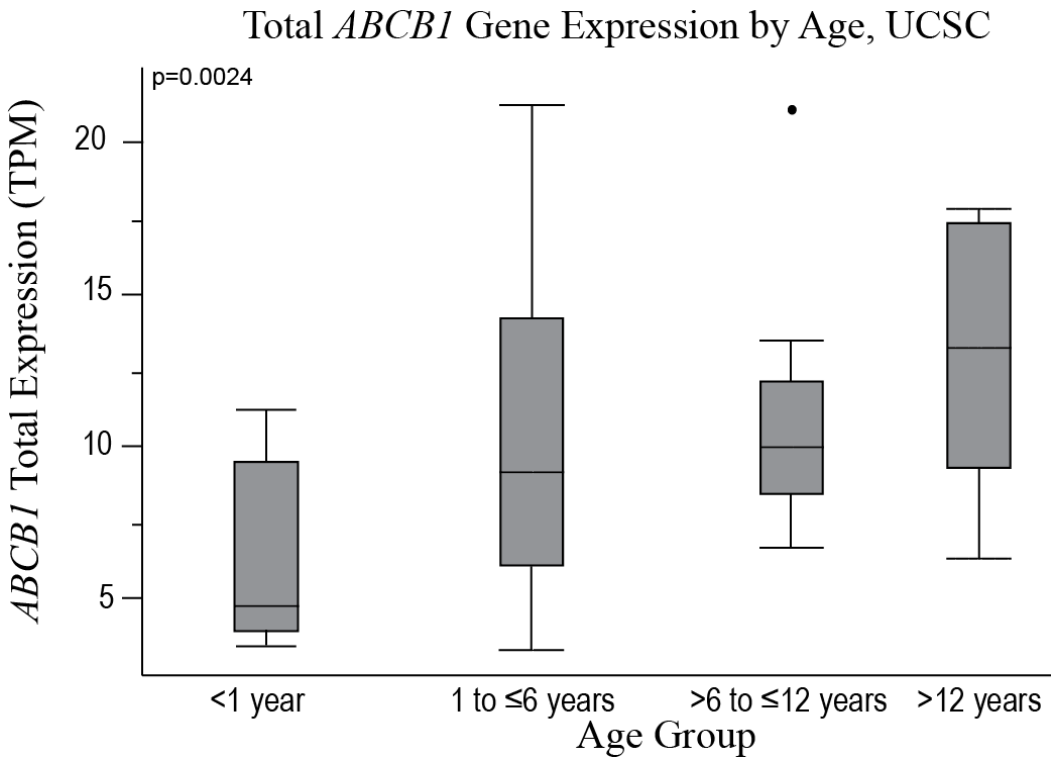

Figure 3. Total Expression (UCSC-based) of *ABCB1* by Age Group.

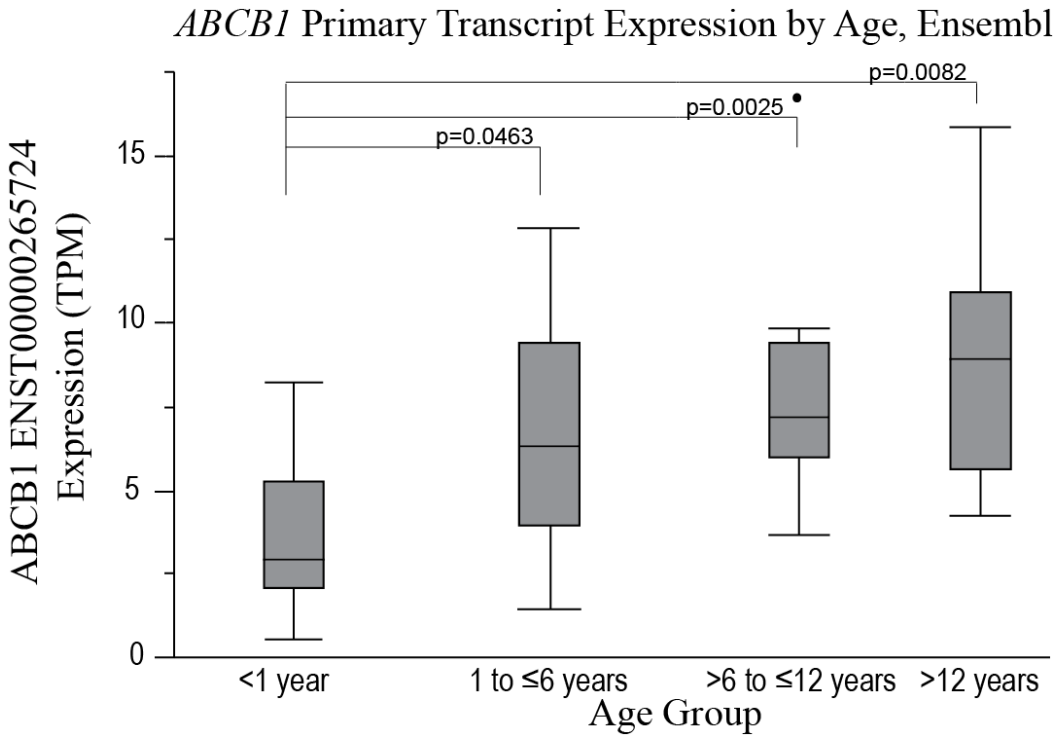

Figure 4. Expression (Ensembl-based) of *ABCB1* Primary Transcript by Age Group. Pairwise comparison p-values presented above box-plots.

The UCSC dataset lists only one transcript for *ABCB1*, so an additional primary transcript analysis was not performed. In the UCSC dataset analysis, *KIF6* NM_001289020 showed significant association with age (p=0.0015) (**Figure 5; Table 5)**. *KIF6* expression was highest in Group 1 (<1 year old), which significantly differed from other groups (**Table 6**). Across all age groups, expression of KIF6 was relatively low (< 5 TPM).

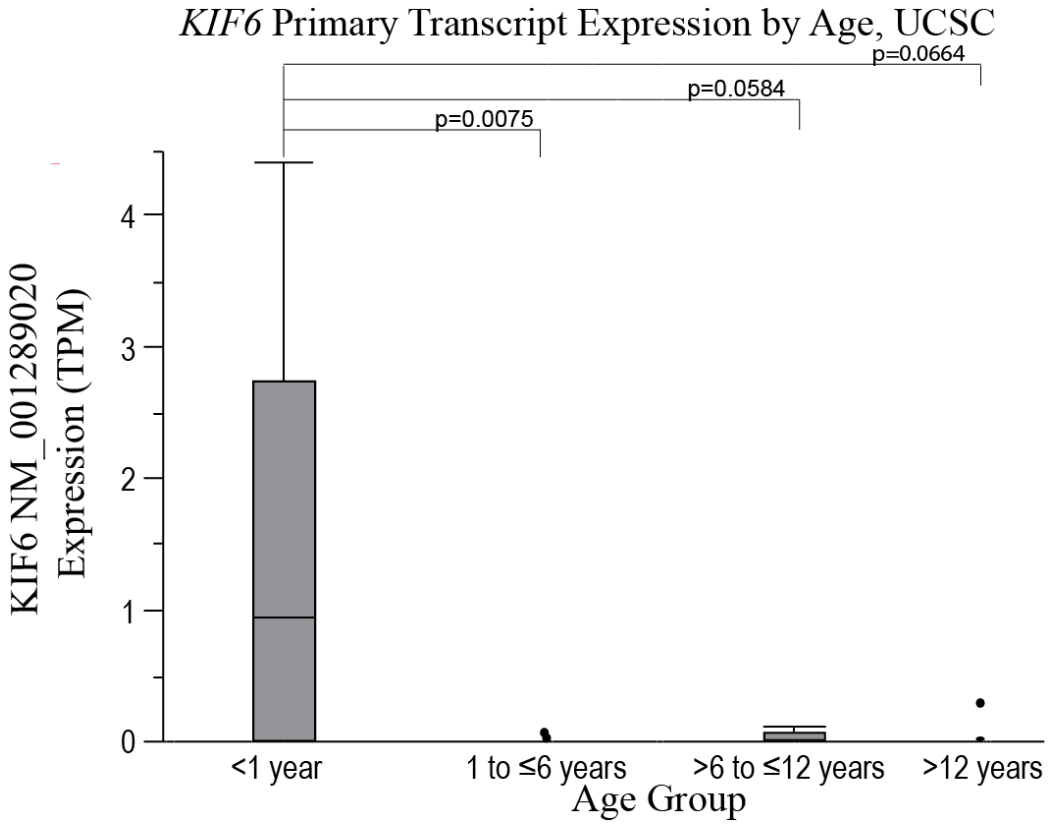

Figure 5. Expression (UCSC-based) of *KIF6* Primary Transcript by Age Group. Pairwise comparison p-values presented above box-plots.

HMGCR and *HNRNPA1* Alternative Transcript Expression

There were no significant differences in the relative expression of alternative and canonical transcripts of *HMGCR* or *HNRNPA1* (HMGCR*Δ*13:HMGCRex13 or HNRNPA1ex8:HNRNPA1*Δ*8) associated with *HMGCR* or *HNRNPA1* genotypes in the general genetic model including the full set of liver tissue samples (**Table 7**). A trend toward an increase

in relative HMGCR*Δ*13 was seen with presence of 'A' alleles in *HMGCR*, but this relationship did not achieve significance (p=0.1015). The Caucasian subgroup general genetic model analysis did not show any significant trends (**Table 8**).

Table 7. Alternative to Canonical Transcript Expression Ratio by Genotype, General Genetic Model.

	Genotype	Mean Ratio (95% CI)	Kruskal Wallis p-value
HMGCR*Δ*13:HMGCRex13	rs3846662 G/G	0.294 (0.19, 0.45)	0.1015
	rs3846662 G/A	0.459 (0.33, 0.64)	
	rs3846662 A/A	0.563 (0.33, 0.97)	
HNRNPA1ex8:HNRNPA1*Δ*8	rs1920045 C/C	0.098 (0.07, 0.14)	0.5425
	rs1920045 C/T	0.149 (0.09, 0.26)	
	rs1920045 T/T	0.112 (0.06, 0.20)	
HMGCR*Δ*13:HMGCRex13	rs1920045 C/C	0.548 (0.42, 0.72)	0.1389
	rs1920045 C/T	0.336 (0.23, 0.50)	
	rs1920045 T/T	0.373(0.17, 0.82)	

Table 8. Alternative to Canonical Transcript Expression Ratio by Genotype, General Genetic Model, Caucasian Subgroup.

	Genotype	Mean Ratio (95% CI)	Kruskal Wallis p-value
HMGCR*Δ*13:HMGCRex13	rs3846662 G/G	0.173 (0.05, 0.60)	0.4187
	rs3846662 G/A	0.273 (0.16, 0.47)	
	rs3846662 A/A	0.313 (0.09, 1.14)	
HNRNPA1ex8:HNRNPA1*Δ*8	rs1920045 C/C	0.087 (0.02, 0.34)	0.9232
	rs1920045 C/T	0.090 (0.05, 0.17)	
	rs1920045 T/T	0.093 (0.00, 31.04)	
HMGCR*Δ*13:HMGCRex13	rs1920045 C/C	0.427 (0.18, 1.01)	0.1359
	rs1920045 C/T	0.187 (0.11, 0.31)	
	rs1920045 T/T	0.213 (0.08, 0.59)	

In the dominant genetic model including the full set of samples, expression of HMGCR*Δ*13:HMGCRex13 approached a significant association with *HNRNPA1* genotype and *HMGCR* genotype (**Table 9**). Presence of at least one 'T' allele in *HNRNPA1* approached an association with lower relative expression of HMGCR*Δ*13 (p=0.0470), while presence of at least one 'A' allele in *HMGCR* approached an association with higher relative expression of

HMGCR⊿13 (p=0.0465) (**Figure 6,7**). In the Caucasian subgroup analyses, no significant associations were found between genotypes and transcript expression ratios in the dominant model; however, the relationship between *HNRNPA1* genotype and relative expression of HMGCR⊿13 was near significance (p=0.0500) (**Table 10**).

Table 9. Alternative to Canonical Transcript Expression Ratio by Genotype, Dominant Genetic Model.

	Genotype	Mean Ratio (95% CI)	Wilcoxon p-value
HMGCR⊿13:HMGCRex13	rs3846662 G/G	0.294 (0.19, 0.45)	0.0465
	rs3846662 G/A, A/A	0.491 (0.38, 0.64)	
HNRNPA1ex8:HNRNPA1⊿8	rs1920045 C/C	0.098 (0.07,0.14)	0.3044
	rs1920045 C/T, T/T	0.139 (0.09, 0.21)	
HMGCR⊿13:HMGCRex13	rs1920045 C/C	0.548 (0.42, 0.72)	0.0470
	rs1920045 C/T, T/T	0.345 (0.25, 0.48)	

Table 10. Alternative to Canonical Transcript Expression Ratio by Genotype, Dominant Genetic Model, Caucasian Subgroup.

	Genotype	Mean Ratio (95% CI)	Wilcoxon p-value
HMGCR⊿13:HMGCRex13	rs3846662 G/G	0.173 (0.05, 0.60)	0.1917
	rs3846662 G/A, A/A	0.287 (0.19, 0.44)	
HNRNPA1ex8:HNRNPA1⊿8	rs1920045 C/C	0.087 (0.02, 0.34)	0.7449
	rs1920045 C/T, T/T	0.090 (0.05, 0.15)	
HMGCR⊿13:HMGCRex13	rs1920045 C/C	0.427 (0.18, 1.01)	0.0500
	rs1920045 C/T, T/T	0.192 (0.13, 0.28)	

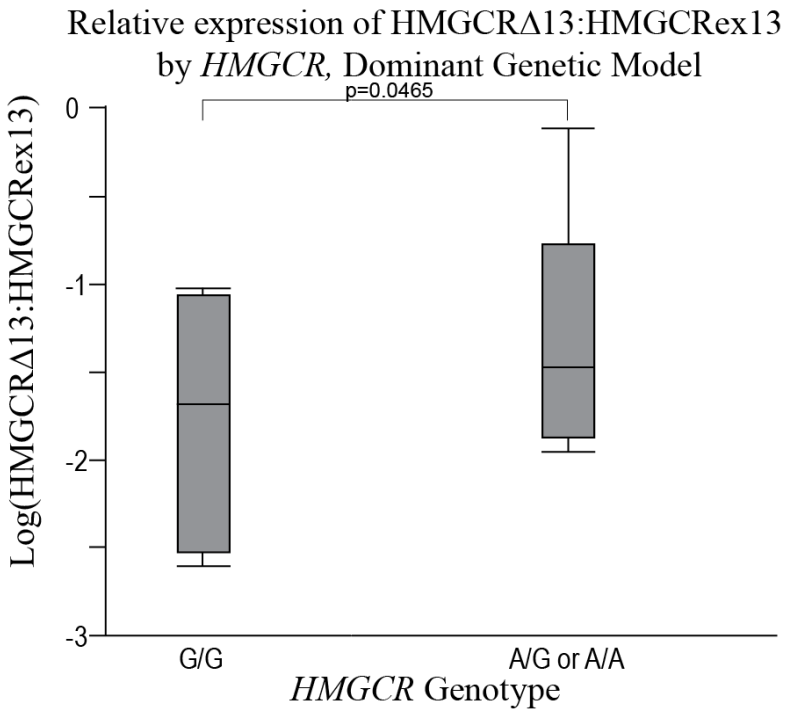

Figure 6. Ratio of Expression of HMGCR*Δ*13 to HMGCRex13 According to *HMGCR* Genotype, Dominant Model.

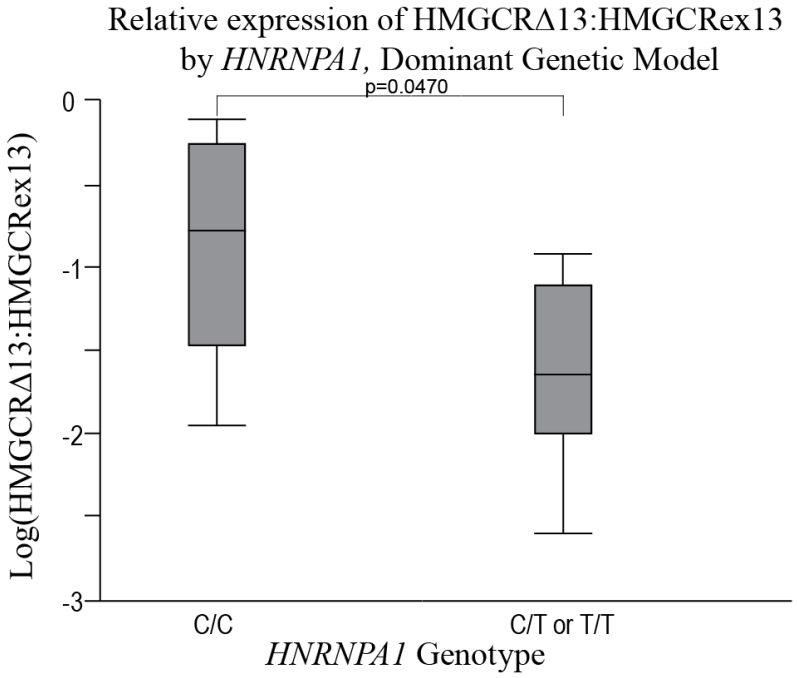

Figure 7. Ratio of Expression of HMGCR*Δ*13 to HMGCRex13 According to *HNRNPA1* Genotype, Dominant Model.

CPR Clinical Analysis

Analysis of the associations between *HMGCR* or *HNRNPA1* genotypes and lipid values in the CPR showed that TC and LDL-C trended toward a significant relationship with the *HNRNPA1* genotype in a general genetic model (p=0.0325 and p=0.0177, respectively) (**Table 11; Figure 8, 9**). The heterozygous group had higher TC and LDL-C than either of the homozygous groups. No other significant associations were found in the analysis of the CPR as a whole.

Table 11. Lipid Panel Values by Genotype, Total CPR.

Gene, SNP	Lipid	Genotype	Mean (95% CI), mg/dL	ANOVA p-value
HMGCR, rs3846662	TC	G/G	238 (227, 250)	0.7608
		G/A	239 (228, 250)	
		A/A	233 (223, 243)	
	LDL-C	G/G	166 (156, 177)	0.1873
		G/A	160 (150, 171)	
		A/A	151 (143, 160)	
	HDL-C	G/G	44 (41, 47)	0.2546
		G/A	46 (43, 49)	
		A/A	48 (44, 52)	
	Triglycerides	G/G	124 (110, 140)	0.4066
		G/A	134 (119, 151)	
		A/A	142 (119, 170)	
HNRNPA1, rs1920045	TC	C/C	232 (221, 243)	0.0325
		C/T	247 (236, 258)	
		T/T	228 (216, 240)	
	LDL-C	C/C	153 (144, 163)	0.0177
		C/T	170 (160, 180)	
		T/T	151 (141, 163)	
	HDL-C	C/C	47 (43, 51)	0.6948
		C/T	46 (44, 48)	
		T/T	45 (41, 49)	
	Triglycerides	C/C	140 (123, 160)	0.5450
		C/T	132 (118, 146)	
		T/T	125 (105, 149)	

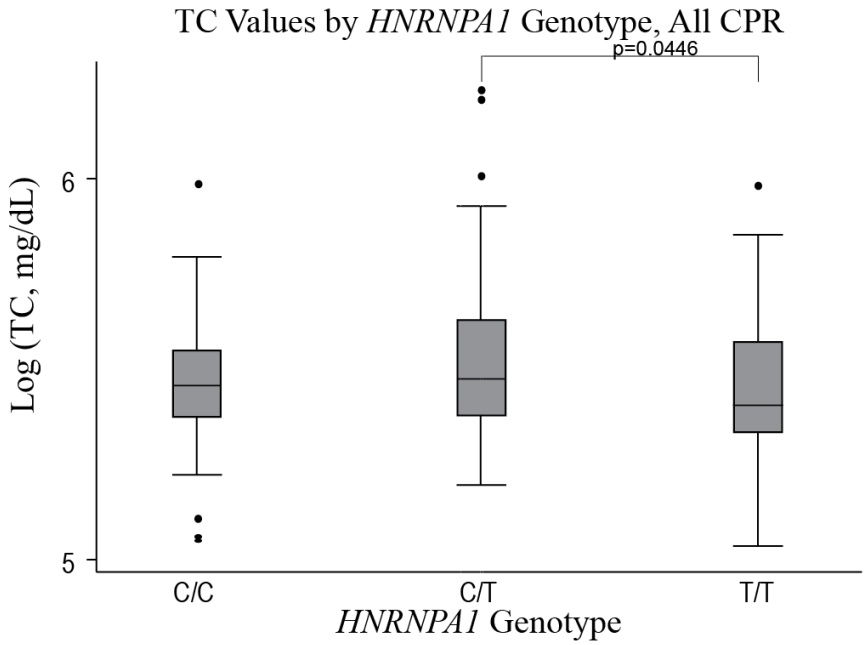

Figure 8. Total Cholesterol Values by *HNRNPA1* Genotype for All CPR Participants. Pairwise comparison p-value presented above box-plots.

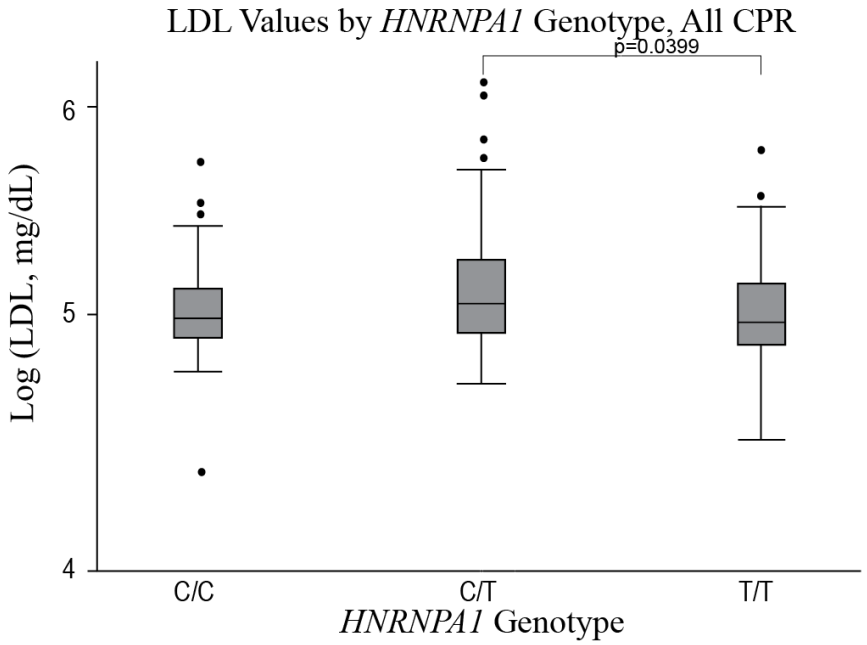

Figure 9. LDL Cholesterol Values by *HNRNPA1* Genotype for All CPR Participants. Pairwise comparison p-value presented above box-plots. There was a missing value for one participant.

After stratification by race, the African American subgroup showed a nearly significant association between *HMGCR* genotype with TC (p=0.0121), as well as with LDL-C (p=0.0309)

(**Table 12; Figures 10, 11**). Lipid levels trended toward an increase with the presence of the 'A' allele. Additionally, *HNRNPA1* genotype showed a near significant association with TC in African Americans (p=0.0464) and LDL-C in Caucasians (p=0.0489) (**Tables 12, 13; Figures 12, 13**). In African Americans, TC was lower in the group homozygous for the 'T' allele compared to the heterozygous group. In Caucasians, LDL-C was highest in those with only one 'T' allele relative to those with either zero or two. After Bonferroni correction for multiple tests, the level of significance for CPR data was set at p<0.002. No relationships met this level of significance. There were no significant associations found between BMI category and either genotype before or after race stratification (**Table 14**).

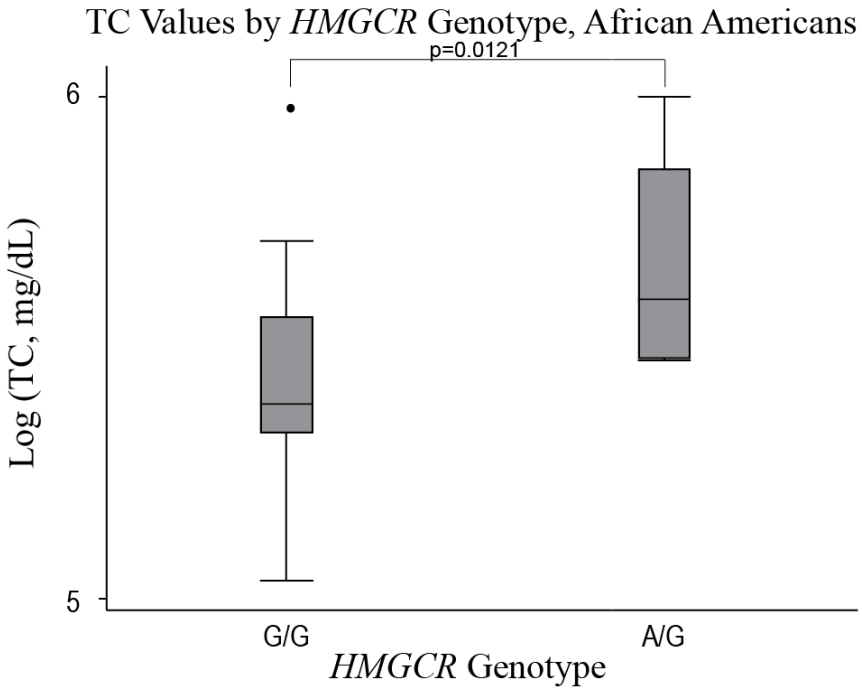

Figure 10. Total Cholesterol Values by *HMGCR* Genotype for African American CPR Participants.

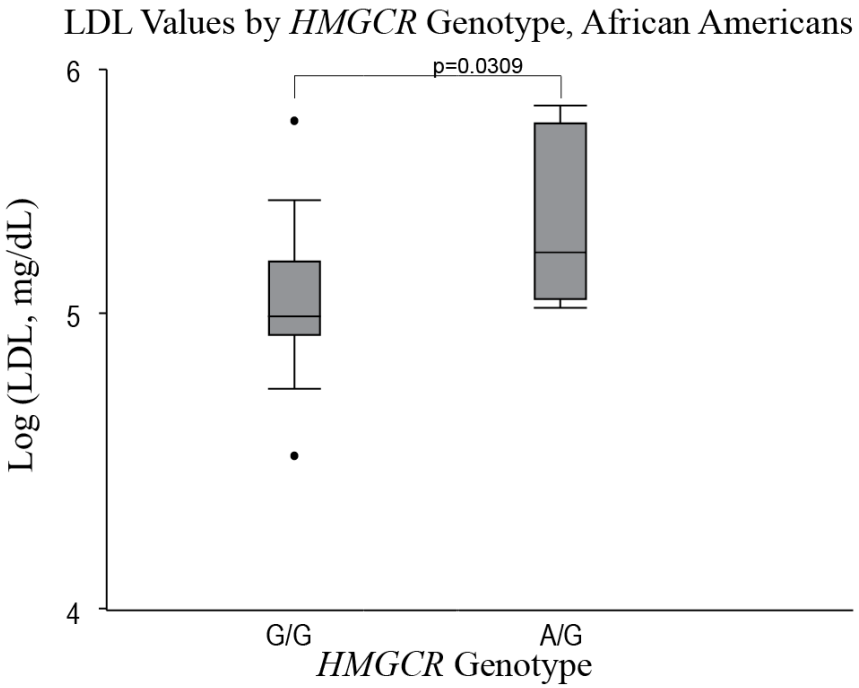

Figure 11. LDL Cholesterol Values by *HMGCR* Genotype for African American CPR Participants.

Table 12. Lipid Panel Values by Genotype, African American Subgroup.

Gene, SNP	Lipid	Genotype	Mean (95% CI), mg/dl	Wilcoxon p-value
HMGCR, rs3846662	TC	G/G	223 (204, 245)	0.0121
		G/A	283 (226, 354)	
		A/A	N/A	
	LDL-C	G/G	156 (138, 176)	0.0309
		G/A	210 (145, 304)	
		A/A	N/A	
	HDL-C	G/G	44 (39, 50)	0.3196
		G/A	52 (40, 69)	
		A/A	N/A	
	Triglycerides	G/G	99 (83, 119)	0.4541
		G/A	79 (32, 192)	
		A/A	N/A	
HNRNPA1, rs1920045	TC	C/C	N/A	0.0464
		C/T	266 (222, 319)	
		T/T	224 (202, 247)	
	LDL-C	C/C	N/A	0.1112
		C/T	193 (145, 258)	
		T/T	156 (137, 178)	
	HDL-C	C/C	N/A	0.7491
		C/T	48 (41, 57)	
		T/T	45 (38, 52)	
	Triglycerides	C/C	N/A	0.2474
		C/T	118 (84, 166)	
		T/T	88 (70, 110)	

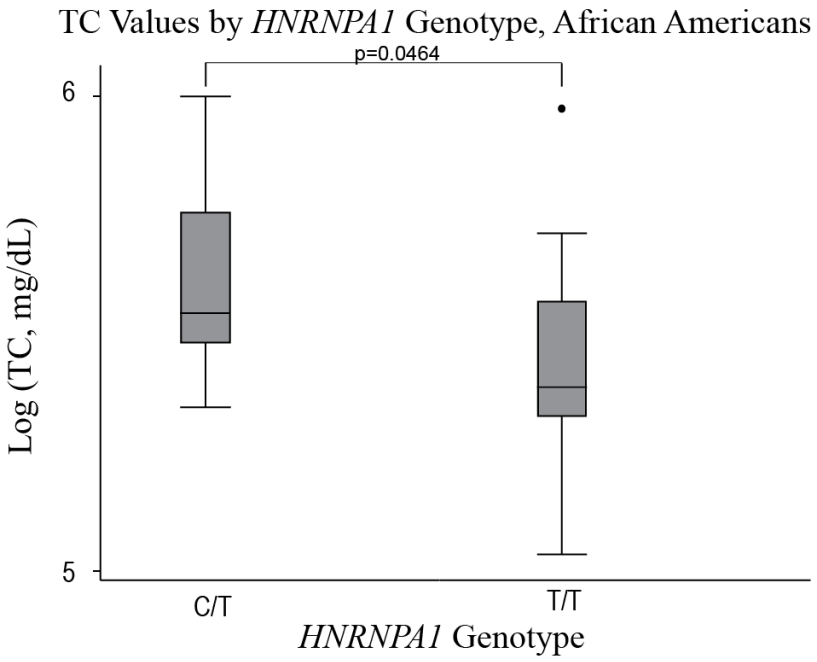

Figure 12. Total Cholesterol Values by *HNRNPA1* Genotype for African American CPR Participants.

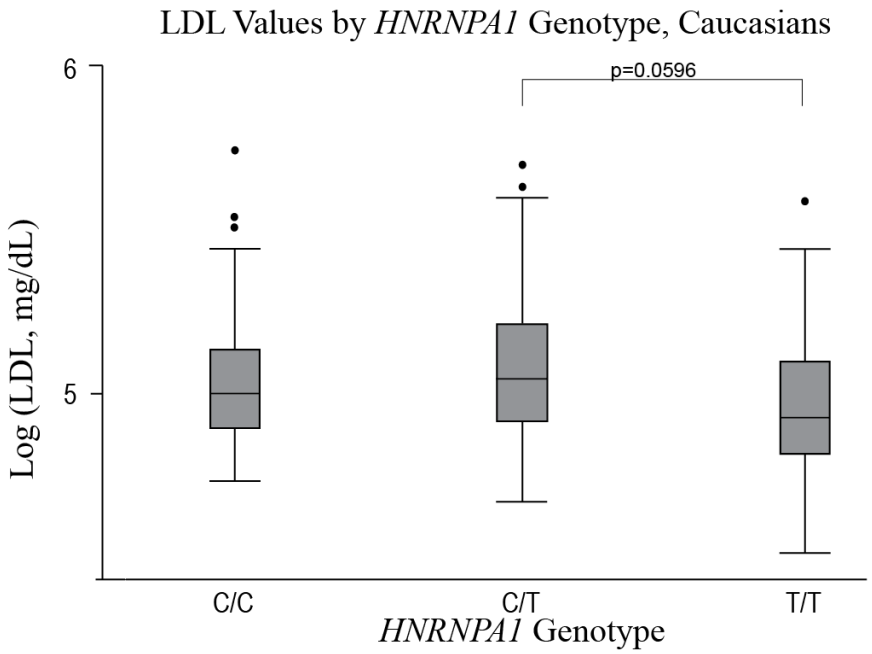

Figure 13. LDL Cholesterol Values by *HNRNPA1* Genotype for Caucasian CPR Participants. Pairwise comparison p-value presented above box-plots.

Table 13. Lipid Panel Values by Genotype, Caucasian Subgroup.

Gene, SNP	Lipid	Genotype	Mean (95% CI), mg/dl	Kruskal Wallis p-value
HMGCR, rs3846662	TC	G/G	241 (226, 256)	0.7182
		G/A	235 (225, 245)	
		A/A	233 (222, 243)	
	LDL-C	G/G	165 (153, 179)	0.3451
		G/A	156 (148, 165)	
		A/A	151 (142, 161)	
	HDL-C	G/G	45 (41, 49)	0.4428
		G/A	45 (43, 48)	
		A/A	47 (43, 51)	
	Triglycerides	G/G	137 (117, 160)	0.7097
		G/A	137 (120, 156)	
		A/A	145 (121, 172)	
HNRNPA1, rs1920045	TC	C/C	234 (223, 244)	0.5431
		C/T	240 (230, 250)	
		T/T	229 (213, 245)	
	LDL-C	C/C	155 (146, 164)	0.0489
		C/T	163 (155, 172)	
		T/T	146 (132, 161)	
	HDL-C	C/C	47 (43, 51)	0.517
		C/T	45 (43, 47)	
		T/T	46 (41, 51)	
	Triglycerides	C/C	140 (122, 160)	0.5859
		C/T	134 (118, 152)	
		T/T	151 (117, 196)	

Table 14. BMI Category by Genotype.

Gene, SNP	Chi-square or Fisher's Exact P-value
All	
HMGCR, rs3846662	0.8702
HNRNPA1, rs1920045*	0.1823
Caucasians	
HMGCR, rs3846662	0.6824
HNRNPA1, rs1920045*	0.2987
African Americans	
HMGCR, rs3846662*	0.0980
HNRNPA1, rs1920045*	1.000

*Fisher's exact test was performed due to counts <5 in some cells

DISCUSSION

It is widely accepted that gene expression changes during childhood growth and development.[1] The study presented here set out to describe pediatric hepatic ontogeny for mRNA expression from 30 genes in pathways associated with cholesterol synthesis and statin response or toxicity, and explore the *in vivo* and *in vitro* presentation of genetic variation within two of these genes, *HMGCR* and *HNRNPA1*, during childhood.

When prenatal samples were included in the analyses, many genes showed a significant change in expression between age groups. This was expected due to the different cellular milieu of the prenatal liver compared to the post-natal liver.[29] The function of the liver changes from hematopoietic (maximal activity around week 15) to gluconeogenic around birth.[30] Each of the genes that showed significant expression changes in the ontogeny analysis with prenatal samples showed, as anticipated from the literature, that the prenatal age group was the source of the significant difference from at least one other group (**Tables B1, B2**). Additionally, the significant gene expression changes found using the Ensembl-based data were similar to those found with the UCSC-based data with a few exceptions that trended toward significance in one dataset and achieved significance in the other: *APOC2*, *CETP*, *COQ2*, and *LPIN1*. These slight differences with similar trends suggest that changes in expression are occurring, but they may not be significant at the mRNA level. Overall, however, the results of the prenatal sample analysis support the biological model that hepatocyte structure and function are dynamic during gestation and immediately following.[29] Since transcriptomic analysis is only a marker for clinically impactful biological changes, further analysis in this area should focus on gene network and proteomic evaluation of the genes identified in this study.

When prenatal samples were excluded to examine differences only between postnatal groups, few individual genes showed significant hepatic expression changes; however, *ABCB1*, which codes for a major drug efflux transporter, MDR1, did show an increase in mRNA expression with age in both the Ensembl-based total and primary transcript expression analyses (**Table 4,6; Figures 2, 5**). MDR1 is thought to transport some statins, as well as multiple non-statin drugs, such as methotrexate, out of the liver.[16,31] A firmer understanding of the trajectory of *ABCB1* expression may contribute to more effective pharmacotherapy for pediatrics. For instance, lower levels of *ABCB1* expression may indicate a time of high hepatic accumulation of statins, and suggest the need for a lower dose. The UCSC database includes only one possible transcript for *ABCB1*, while the Ensembl dataset includes 10. Since *ABCB1* in the UCSC dataset showed a trend toward a significant increase in expression with age that did not achieve significance, further confirmation of the trend identified with the Ensembl dataset should be performed with a larger number of samples, focusing on the transcripts covered. The ontogenetic pattern found in this study supports the trend described by Mooij, *et al*. and further differentiates between the expression levels at particular developmental stages.[19] A proteomic study of hepatic MDR1 expression and activity could elucidate the functional implications of this mRNA expression change. Furthermore, although few individual genes showed significant expression changes with age, the sum of individually small changes may be acting in a network to affect the pathways associated with cholesterol metabolism and statin therapeutic response and toxicity. For this reason, gene network analysis should be performed with the RNA-seq dataset as the next step in examination of this dataset.

The associations between *HMGCR* (rs3846662) or *HNRNPA1* (rs1920045) genotype and the relative expression of alternative and canonical transcripts of *HMGCR* and *HNRNPA1* were

not found to be statistically significant in the pediatric liver samples. Burkhardt, *et al*. described how HMGCR*Δ*13 relative expression was increased in cells carrying an *HMGCR* 'A' allele in LCLs. Our results showed a trend of increased relative HMGCR*Δ*13 expression in pediatric liver samples that did not achieve statistical significance in either the general genetic or dominant *HMGCR* model. Yu, *et al*. described that the 'T' allele upstream of *HNRNPA1* is associated with altered constitutive expression of HNRNPA1ex8 in LCLs (Yu, *et al*. Supplemental Figure S6-A). Neither the general genetic model nor the dominant model supported an expression association between *HNRNPA1* genotype and relative expression of HNRNPA1ex8 in pediatric livers (**Tables 7, 9**). It was hypothesized that the 'T' allele should be expected to be associated with a decrease in constitutive relative HMGCR*Δ*13. No significant expression differences were detected between *HNRNPA1* genotype groups in the general genetic model, suggesting that, in pediatric hepatocytes, the associations are not strong. The dominant *HNRNPA1* genetic model, however, did show a trend toward a significant expression change, with decreased HMGCR*Δ*13 expression in the presence of one or two 'T' alleles, in line with the hypothesized effect. This result suggests that the hypothesized relationship between rs1920045 and *HMGCR* expression may be present in the pediatric population, but should be confirmed in a larger set of samples. The allele frequencies for both genotypes differ by race, which led to the decision to stratify the results by this factor. The dbSNP published frequencies of the *HMGCR* 'G' alleles in Caucasians and African Americans (population AoD) are 0.40 and 0.86, respectively. The dbSNP published frequencies of *HNRNPA1* 'C' alleles in Caucasians and African Americans (population AoD) are 0.63 and 0.29, respectively. In the Caucasian subgroup analyses, no significant associations between genotype and expression were found in either model. Analysis of the African American subgroup was not possible due to limited sample size. There are some limitations to our

genotype-associated expression analysis. The small sample size may have caused the race stratification analysis to be underpowered, thus masking significant trends. Additionally, the samples in this study spanned multiple developmental ages, however, small samples in each age/genotype group precluded stratification analysis. Preliminary investigation into the relative expression of HMGCRΔ13 across developmental ages trended toward, but did not reach significance, showing the need for further studies on the relationship between age and this expression (data not shown). An expansion of the sample size to include better representation of racial groups and developmental ages should be pursued in order to further evaluate the relationship between expression and genotype in children.

Evaluation of the relationships between *HMGCR* (rs3846662) and *HNRNPA1* (rs1920045) genotypes and *in vivo* lipid levels revealed interesting associations. Based on adult studies and cell models, the *HMGCR* 'A' and *HNRNPA1* 'C' alleles were anticipated to be associated with lower baseline total and LDL cholesterol values.[2,24] In the CPR sample evaluated as a whole, no association was seen between baseline cholesterol values and *HMGCR* genotype. Near significant associations between *HNRNPA1* genotype and TC or LDL-C were found. In both cases, the heterozygous group had higher TC and LDL-C values than either of the homozygous groups. Since the group showing different expression was heterozygous, this finding was inconclusive. Once the cohort was stratified by race, some of the associations between lipid panel values and genotype changed. The Caucasian subgroup analysis did not reveal any significant relationships between genotypes and age, although the relationship between LDL-C and *HNRNPA1* genotype approached significance, with the same pattern of highest LDL-C values in the heterozygous group. In the African American subgroup, the relationships between *HMGCR* genotype and TC or LDL-C trended toward significance and

average levels increased with the presence of one ‘A’ allele. This was opposite of the expected relationship. Additionally, a trend toward a significant decrease in TC with the presence of the *HNRNPA1* ‘T’ allele was noted. After the Bonferroni correction for multiple tests, no relationships met the criteria for significance, yet this is considered to be an overly-conservative adjustment for significance. The data showed preliminary evidence for interesting trends between *HMGCR* or *HNRNPA1* genotypes and lipid levels in children and revealed that race may be confounding the genotype-phenotype relationship. This information indicated that further studies should focus on ensuring adequate representation of racial groups when characterizing the clinical implications of these SNPs in pediatrics. An additional limitation in the evaluation of *in vivo* lipid levels was that the study group overall had higher lipid levels than average, and there was no comparison to a group of children with “acceptable” lipid levels. Further investigations into this, and into trends in lipid levels over time, may better elucidate the relationship between lipid levels and these genotypes. One additional limitation is in the race stratification. Using self-reported race may not be as accurate as identifying race by genotyping, and including individuals reporting Hispanic ethnicity within their respective racial group, without stratification, may mask some relationships that could be present.

In conclusion, this study confirmed the presence of dynamic gene expression in the liver during childhood development; however the number of genes with expression changes after birth was less than anticipated. For this study, and many others, mRNA was used as a surrogate marker to investigate downstream changes, such as protein expression. Since mRNA is not translated at a universal rate and can be differentially degraded, the lack of mRNA expression changes in the pediatric hepatocytes may not actually indicate constant expression of transporter proteins or enzymes. Future studies should focus on protein activity changes throughout

development. In particular, the implications of *ABCB1* mRNA expression increasing with age should be evaluated on a protein level, as the MDR1 transporter is involved with multiple medications, and this knowledge could contribute to pharmacokinetic models.

Furthermore, the information in this study suggests that the impact of genetic variation in pediatrics may not mimic that of adult relationships. Genotype-phenotype relationships that are significant in adult populations may not be significant in children, or the relationships may be altered. Our study showed that the expression changes associated with two SNPs in LCLs were not significant in pediatric hepatocytes, but certain relationships trended toward significance. *In vivo*, the impact of these SNPs appeared to be confounded by race. Relationships approaching significance appeared after stratification, including two with phenotypes opposite of what had been hypothesized.

The challenges of establishing adequate sample sizes for full genomic studies in the pediatric setting highlights the importance of maximizing that data from each study. The indication that race and age may confound phenotypic presentation in individuals from this study points to the need to ensure adequate representation of different races and developmental stages in future pediatric pharmacogenomic studies.

When possible, data on established genotype-phenotype relationships from adult studies should be utilized to inform therapeutic interventions in children; however, only studies conducted in pediatric populations will accurately capture variability attributable to maturity, which is not evident from adult studies.

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APPENDIX A

List of Gene Abbreviations

ABCB1 – ATP-Binding Cassette, Sub-Family B, Member 1

AGTR1 – Angiotensin II Receptor, Type 1

AMPD1 – Adenosine Monophosphate Deaminase 1

APOC1 – Apolipoprotein C-I

APOC2 – Apolipoprotein C-II

APOE – Apolipoprotein E

ATP2B1 – ATPase, Ca++ Transporting, Plasma Membrane 1

CETP – Cholesterol Ester Transfer Protein, plasma

CLMN – Calmin

CPT2 – Carnitine Palmitoyltransferase 2

COQ2 – Coenzyme Q2 4-hydroxybenzoate Polyprenyltransferase

CYP7A1 – Cytochrome P450, Family 7, Subfamily A, Polypeptide 1

DMPK – Dystrophia Myotonica-protein Kinase

DNAJC5B – DnaJ(Hsp40) homolog, Subfamily C, Member 5 Beta

GATM – Glycine Amidinotransferase

HMGCR – 3-Hydroxy-3-methylglutaryl CoA Reductase

HNRNPA1 – Heterogeneous Nuclear Ribonucleoprotein A1

HTR3B – 5-Hydroxytryptamine Receptor 3B, Ionotropic

HTR7 – 5-Hydroxytrptamine Receptor 7, Adenylate Cyclase-coupled

KIF6 – Kinesin Family Member 6

LDLR – Low Density Lipoprotein Receptor

LPA – Lipoprotein, Lp(a)

LPIN1 – Lipin 1

MDR1* – Multidrug Resistance Protein 1

MYLIP – Myosin Regulatory Light Chain Interacting Protein

NOS3 – Nitric Oxide Synthase 3

PCSK9 – Proprotein Convertase Subtilisin/Kexin Type 9

PYGM –Phosphorylase, Glycogen, Muscle

RYR1 – Ryanodine Receptor 1

SLC10A1 – Solute Carrier Family 10, Member 1

SLCO1B1 – Solute Carrier Organic Anion Transporter Family, Member 1B1

*Protein, not gene

APPENDIX B

Table B1. Pairwise Comparison Results for Total Gene Expression with Prenatal Samples by Age Group Analysis.

	Ensembl			UCSC		
Gene	**Comparison**		**p-value**	**Comparison**		**p-value**
ABCB1	Group 2	Group 0	0.0013	Group 2	Group 0	0.0003
ABCB1	Group 3	Group 0	0.0006	Group 3	Group 0	0.0006
ABCB1	Group 3	Group 1	0.0093	Group 1	Group 0	0.0009
ABCB1	Group 2	Group 1	0.0356	Group 3	Group 1	0.0275
ABCB1	Group 4	Group 1	0.0131	Group 4	Group 1	0.0163
ABCB1	Group 4	Group 0	0.0041	Group 4	Group 0	0.0041
ABCB1	Group 1	Group 0	0.1232	Group 2	Group 1	0.1425
ABCB1	Group 4	Group 3	0.7113	Group 4	Group 2	0.5622
ABCB1	Group 4	Group 2	0.8211	Group 4	Group 3	0.6198
ABCB1	Group 3	Group 2	0.9399	Group 3	Group 2	0.9692
APOC1	Group 3	Group 0	0.0006	Group 2	Group 0	0.0003
APOC1	Group 2	Group 0	0.0125	Group 3	Group 0	0.0006
APOC1	Group 4	Group 0	0.0056	Group 1	Group 0	0.0045
APOC1	Group 1	Group 0	0.0805	Group 4	Group 0	0.0041
APOC1	Group 3	Group 2	0.5540	Group 3	Group 2	0.4140
APOC1	Group 3	Group 1	0.9657	Group 4	Group 2	0.9999
APOC1	Group 4	Group 2	0.9999	Group 3	Group 1	1.0000
APOC1	Group 4	Group 1	0.9528	Group 4	Group 3	0.8654
APOC1	Group 2	Group 1	0.9395	Group 4	Group 1	0.7547
APOC1	Group 4	Group 3	0.7113	Group 2	Group 1	0.4441
APOC2				Group 2	Group 0	0.0034
APOC2				Group 1	Group 0	0.0029
APOC2				Group 3	Group 0	0.0042
APOC2				Group 4	Group 0	0.0041
APOC2				Group 3	Group 2	0.9131
APOC2				Group 4	Group 2	0.9778
APOC2				Group 3	Group 1	0.9865
APOC2				Group 4	Group 3	0.9205
APOC2				Group 2	Group 1	0.7835
APOC2				Group 4	Group 1	0.6719
ATP2B1	Group 3	Group 2	0.9986	Group 3	Group 2	0.4411
ATP2B1	Group 3	Group 1	0.9963	Group 4	Group 2	0.9853
ATP2B1	Group 2	Group 1	0.9275	Group 3	Group 1	0.7436
ATP2B1	Group 4	Group 3	0.8315	Group 4	Group 3	0.4327
ATP2B1	Group 4	Group 2	0.8212	Group 4	Group 1	0.1991
ATP2B1	Group 4	Group 1	0.6719	Group 2	Group 1	0.1179
ATP2B1	Group 4	Group 0	0.0041	Group 4	Group 0	0.0041
ATP2B1	Group 1	Group 0	0.0012	Group 1	Group 0	0.0180
ATP2B1	Group 3	Group 0	0.0008	Group 3	Group 0	0.0006
ATP2B1	Group 2	Group 0	0.0003	Group 2	Group 0	0.0003
CETP	Group 4	Group 2	0.8362	Group 4	Group 2	0.6023
CETP	Group 4	Group 3	0.9205	Group 4	Group 3	0.6662
CETP	Group 3	Group 2	0.9609	Group 4	Group 1	0.7926
CETP	Group 4	Group 1	0.9958	Group 3	Group 2	0.9959
CETP	Group 3	Group 1	1.0000	Group 3	Group 1	0.9979
CETP	Group 2	Group 1	0.9141	Group 2	Group 1	0.9747
CETP	Group 4	Group 0	0.0041	Group 4	Group 0	0.4138
CETP	Group 1	Group 0	0.0015	Group 1	Group 0	0.0083
CETP	Group 3	Group 0	0.0008	Group 3	Group 0	0.0052
CETP	Group 2	Group 0	0.0007	Group 2	Group 0	0.0034

Table B1. Pairwise Comparison Results for Total Gene Expression with Prenatal Samples Continued.

	Ensembl			UCSC		
Gene	**Comparison**		**p-value**	**Comparison**		**p-value**
COQ2	Group 3	Group 1	0.3617			
COQ2	Group 3	Group 2	0.4409			
COQ2	Group 4	Group 1	0.7547			
COQ2	Group 4	Group 2	0.9015			
COQ2	Group 2	Group 1	1.0000			
COQ2	Group 4	Group 3	0.9985			
COQ2	Group 4	Group 0	0.3610			
COQ2	Group 3	Group 0	0.1024			
COQ2	Group 1	Group 0	0.0005			
COQ2	Group 2	Group 0	0.0004			
CYP7A1	Group 1	Group 0	0.0008	Group 1	Group 0	0.0009
CYP7A1	Group 3	Group 0	0.0007	Group 3	Group 0	0.0034
CYP7A1	Group 2	Group 0	0.0321	Group 4	Group 0	0.0132
CYP7A1	Group 4	Group 0	0.0082	Group 2	Group 0	0.0976
CYP7A1	Group 3	Group 2	0.2461	Group 3	Group 2	0.5369
CYP7A1	Group 4	Group 2	0.9220	Group 4	Group 2	0.8889
CYP7A1	Group 4	Group 3	0.9417	Group 4	Group 3	0.9828
CYP7A1	Group 4	Group 1	0.3882	Group 4	Group 1	0.2934
CYP7A1	Group 3	Group 1	0.2828	Group 3	Group 1	0.1857
CYP7A1	Group 2	Group 1	0.0072	Group 2	Group 1	0.0054
DMPK	Group 4	Group 3	0.8319	Group 4	Group 3	0.9947
DMPK	Group 4	Group 2	1.0000	Group 4	Group 2	0.8898
DMPK	Group 4	Group 1	0.4948	Group 3	Group 2	0.5540
DMPK	Group 3	Group 2	0.5827	Group 2	Group 1	0.4055
DMPK	Group 2	Group 1	0.5104	Group 1	Group 0	0.2293
DMPK	Group 4	Group 0	0.0077	Group 4	Group 1	0.0780
DMPK	Group 3	Group 1	0.0420	Group 4	Group 0	0.0041
DMPK	Group 1	Group 0	0.0258	Group 3	Group 1	0.0151
DMPK	Group 3	Group 0	0.0006	Group 2	Group 0	0.0061
DMPK	Group 2	Group 0	0.0013	Group 3	Group 0	0.0006
HMGCR	Group 2	Group 1	1.0000	Group 2	Group 1	0.9967
HMGCR	Group 4	Group 1	1.0000	Group 3	Group 2	1.0000
HMGCR	Group 3	Group 1	1.0000	Group 4	Group 1	1.0000
HMGCR	Group 3	Group 2	1.0000	Group 3	Group 1	1.0000
HMGCR	Group 4	Group 2	1.0000	Group 4	Group 3	0.9947
HMGCR	Group 4	Group 3	1.0000	Group 4	Group 2	0.9778
HMGCR	Group 4	Group 0	0.0077	Group 4	Group 0	0.0139
HMGCR	Group 2	Group 0	0.0023	Group 3	Group 0	0.0008
HMGCR	Group 3	Group 0	0.0006	Group 2	Group 0	0.0023
HMGCR	Group 1	Group 0	0.0005	Group 1	Group 0	0.0006
HNRNPA1	Group 4	Group 1	1.0000	Group 3	Group 2	1.0000
HNRNPA1	Group 3	Group 2	1.0000	Group 4	Group 2	0.9999
HNRNPA1	Group 4	Group 2	1.0000	Group 4	Group 3	0.9947
HNRNPA1	Group 3	Group 1	1.0000	Group 3	Group 1	0.9907
HNRNPA1	Group 2	Group 1	0.9997	Group 2	Group 1	0.9502
HNRNPA1	Group 4	Group 3	0.9976	Group 4	Group 1	0.8280
HNRNPA1	Group 4	Group 0	0.0041	Group 4	Group 0	0.0041
HNRNPA1	Group 3	Group 0	0.0006	Group 2	Group 0	0.0023
HNRNPA1	Group 1	Group 0	0.0005	Group 3	Group 0	0.0006
HNRNPA1	Group 2	Group 0	0.0004	Group 1	Group 0	0.0005

Table B1. Pairwise Comparison Results for Total Gene Expression with Prenatal Samples Continued.

	Ensembl			UCSC		
Gene	**Comparison**		**p-value**	**Comparison**		**p-value**
KIF6	Group 4	Group 3	0.9985	Group 3	Group 2	0.8655
KIF6	Group 3	Group 2	0.9998	Group 4	Group 2	0.9988
KIF6	Group 4	Group 2	0.9999	Group 4	Group 3	0.8002
KIF6	Group 4	Group 1	0.1004	Group 4	Group 1	0.1241
KIF6	Group 4	Group 0	0.0041	Group 3	Group 1	0.1157
KIF6	Group 3	Group 1	0.0204	Group 2	Group 1	0.0412
KIF6	Group 2	Group 1	0.0245	Group 4	Group 0	0.0036
KIF6	Group 3	Group 0	0.0006	Group 1	Group 0	0.0035
KIF6	Group 1	Group 0	0.0007	Group 3	Group 0	0.0006
KIF6	Group 2	Group 0	0.0003	Group 2	Group 0	0.0002
LPIN1				Group 2	Group 0	0.0003
LPIN1				Group 3	Group 0	0.0008
LPIN1				Group 1	Group 0	0.0023
LPIN1				Group 2	Group 1	0.0357
LPIN1				Group 4	Group 0	0.0836
LPIN1				Group 3	Group 1	0.2592
LPIN1				Group 4	Group 1	0.9996
LPIN1				Group 3	Group 2	0.8974
LPIN1				Group 4	Group 3	0.3886
LPIN1				Group 4	Group 2	0.1896
MYLIP	Group 4	Group 3	1.0000	Group 4	Group 3	1.0000
MYLIP	Group 3	Group 2	1.0000	Group 3	Group 2	1.0000
MYLIP	Group 3	Group 1	0.9374	Group 4	Group 2	0.9122
MYLIP	Group 2	Group 1	0.9274	Group 4	Group 1	0.7921
MYLIP	Group 4	Group 1	0.8747	Group 3	Group 1	0.6385
MYLIP	Group 4	Group 2	0.8774	Group 2	Group 1	0.6053
MYLIP	Group 4	Group 0	0.0041	Group 4	Group 0	0.0041
MYLIP	Group 3	Group 0	0.0006	Group 1	Group 0	0.0009
MYLIP	Group 1	Group 0	0.0005	Group 3	Group 0	0.0006
MYLIP	Group 2	Group 0	0.0003	Group 2	Group 0	0.0003
RYR1	Group 4	Group 3	1.0000	Group 3	Group 2	0.9920
RYR1	Group 4	Group 2	0.9614	Group 4	Group 3	0.9856
RYR1	Group 3	Group 2	0.8596	Group 4	Group 2	0.8949
RYR1	Group 2	Group 1	0.8352	Group 4	Group 1	0.4009
RYR1	Group 4	Group 1	0.3459	Group 1	Group 0	0.3445
RYR1	Group 1	Group 0	0.0862	Group 4	Group 0	0.1008
RYR1	Group 3	Group 1	0.1070	Group 2	Group 1	0.1800
RYR1	Group 4	Group 0	0.0185	Group 3	Group 1	0.1315
RYR1	Group 2	Group 0	0.0186	Group 3	Group 0	0.0057
RYR1	Group 3	Group 0	0.0029	Group 2	Group 0	0.0026

Table B2. Pairwise Comparison Results for Primary Transcript Expression with Prenatal Samples by Age Group Analysis.

Transcript	Ensembl Comparison		p-value	UCSC Comparison	p-value
ABCB1 ENST00000265724	Group 2	Group 0	0.0013		
ABCB1 ENST00000265724	Group 3	Group 0	0.0006		
ABCB1 ENST00000265724	Group 3	Group 1	0.0040		
ABCB1 ENST00000265724	Group 4	Group 1	0.0131		
ABCB1 ENST00000265724	Group 4	Group 0	0.0041		
ABCB1 ENST00000265724	Group 2	Group 1	0.0707		
ABCB1 ENST00000265724	Group 1	Group 0	0.2043		
ABCB1 ENST00000265724	Group 4	Group 2	0.7368		
ABCB1 ENST00000265724	Group 3	Group 2	0.9511		
ABCB1 ENST00000265724	Group 4	Group 3	0.9591		
APOC1 ENST00000252491	Group 3	Group 0	0.0006		
APOC1 ENST00000252491	Group 2	Group 0	0.0125		
APOC1 ENST00000252491	Group 4	Group 0	0.0056		
APOC1 ENST00000252491	Group 1	Group 0	0.0805		
APOC1 ENST00000252491	Group 3	Group 2	0.5254		
APOC1 ENST00000252491	Group 3	Group 1	0.9557		
APOC1 ENST00000252491	Group 4	Group 2	0.9995		
APOC1 ENST00000252491	Group 4	Group 1	0.9528		
APOC1 ENST00000252491	Group 2	Group 1	0.9502		
APOC1 ENST00000252491	Group 4	Group 3	0.7113		
CETP ENST00000200676	Group 4	Group 2	0.9909		
CETP ENST00000200676	Group 4	Group 3	0.9991		
CETP ENST00000200676	Group 2	Group 1	1.0000		
CETP ENST00000200676	Group 3	Group 2	1.0000		
CETP ENST00000200676	Group 4	Group 1	1.0000		
CETP ENST00000200676	Group 3	Group 1	0.9923		
CETP ENST00000200676	Group 4	Group 0	0.0041		
CETP ENST00000200676	Group 3	Group 0	0.0013		
CETP ENST00000200676	Group 1	Group 0	0.0015		
CETP ENST00000200676	Group 2	Group 0	0.0017		
CLMN ENST00000556454	Group 3	Group 0	0.0008		
CLMN ENST00000556454	Group 1	Group 0	0.0015		
CLMN ENST00000556454	Group 2	Group 0	0.0038		
CLMN ENST00000556454	Group 4	Group 0	0.0139		
CLMN ENST00000556454	Group 3	Group 2	1.0000		
CLMN ENST00000556454	Group 4	Group 2	1.0000		
CLMN ENST00000556454	Group 4	Group 3	0.9998		
CLMN ENST00000556454	Group 3	Group 1	0.9557		
CLMN ENST00000556454	Group 2	Group 1	0.9596		
CLMN ENST00000556454	Group 4	Group 1	0.9351		

Table B2. Pairwise Comparison Results for Primary Transcript Expression Continued with Prenatal Samples.

	Ensembl			UCSC		
Transcript	**Comparison**		**p-value**	**Comparison**		**p-value**
COQ2 ENST00000311469	Group 3	Group 1	0.1405			
COQ2 ENST00000311469	Group 3	Group 2	0.2042			
COQ2 ENST00000311469	Group 4	Group 2	0.2087			
COQ2 ENST00000311469	Group 4	Group 1	0.1696			
COQ2 ENST00000311469	Group 2	Group 1	0.9994			
COQ2 ENST00000311469	Group 4	Group 3	1.0000			
COQ2 ENST00000311469	Group 4	Group 0	0.5266			
COQ2 ENST00000311469	Group 3	Group 0	0.4695			
COQ2 ENST00000311469	Group 2	Group 0	0.0033			
COQ2 ENST00000311469	Group 1	Group 0	0.0006			
DMPK ENST00000600757 or NM_001081563	Group 4	Group 3	0.9997	Group 4	Group 3	0.9995
DMPK ENST00000600757 or NM_001081563	Group 3	Group 2	0.9999	Group 4	Group 2	0.9997
DMPK ENST00000600757 or NM_001081563	Group 4	Group 2	1.0000	Group 3	Group 2	0.9997
DMPK ENST00000600757 or NM_001081563	Group 4	Group 1	0.3770	Group 1	Group 0	0.6052
DMPK ENST00000600757 or NM_001081563	Group 3	Group 1	0.2505	Group 4	Group 1	0.1373
DMPK ENST00000600757 or NM_001081563	Group 2	Group 1	0.2387	Group 4	Group 0	0.0184
DMPK ENST00000600757 or NM_001081563	Group 1	Group 0	0.0923	Group 2	Group 1	0.0965
DMPK ENST00000600757 or NM_001081563	Group 4	Group 0	0.0131	Group 3	Group 1	0.0340
DMPK ENST00000600757 or NM_001081563	Group 3	Group 0	0.0025	Group 3	Group 0	0.0016
DMPK ENST00000600757 or NM_001081563	Group 2	Group 0	0.0014	Group 2	Group 0	0.0042
HMGCR ENST00000287936 or NM_000859	Group 3	Group 2	0.9999	Group 4	Group 3	0.9828
HMGCR ENST00000287936 or NM_000859	Group 2	Group 1	1.0000	Group 4	Group 2	0.9995
HMGCR ENST00000287936 or NM_000859	Group 3	Group 1	1.0000	Group 4	Group 1	0.9998
HMGCR ENST00000287936 or NM_000859	Group 4	Group 1	0.9998	Group 2	Group 1	0.9984
HMGCR ENST00000287936 or NM_000859	Group 4	Group 2	0.9972	Group 3	Group 2	0.9968
HMGCR ENST00000287936 or NM_000859	Group 4	Group 3	0.9947	Group 3	Group 1	0.9374
HMGCR ENST00000287936 or NM_000859	Group 4	Group 0	0.0056	Group 4	Group 0	0.0104
HMGCR ENST00000287936 or NM_000859	Group 3	Group 0	0.0006	Group 1	Group 0	0.0019
HMGCR ENST00000287936 or NM_000859	Group 2	Group 0	0.0019	Group 3	Group 0	0.0006
HMGCR ENST00000287936 or NM_000859	Group 1	Group 0	0.0005	Group 2	Group 0	0.0015
HNRNPA1 ENST00000547276 or NM_002136	Group 4	Group 1	0.9981	Group 3	Group 2	0.9959
HNRNPA1 ENST00000547276 or NM_002136	Group 3	Group 2	0.9997	Group 4	Group 2	0.9947
HNRNPA1 ENST00000547276 or NM_002136	Group 4	Group 2	1.0000	Group 3	Group 1	0.9907

Table B2. Pairwise Comparison Results for Primary Transcript Expression Continued with Prenatal Samples.

	Ensembl			UCSC		
Transcript	**Comparison**		**p-value**	**Comparison**		**p-value**
HNRNPA1 ENST00000547276 or NM_002136	Group 4	Group 3	1.0000	Group 4	Group 3	0.9418
HNRNPA1 ENST00000547276 or NM_002136	Group 2	Group 1	1.0000	Group 4	Group 1	0.8280
HNRNPA1 ENST00000547276 or NM_002136	Group 3	Group 1	0.9989	Group 2	Group 1	0.8270
HNRNPA1 ENST00000547276 or NM_002136	Group 4	Group 0	0.0041	Group 4	Group 0	0.0041
HNRNPA1 ENST00000547276 or NM_002136	Group 3	Group 0	0.0010	Group 3	Group 0	0.0010
HNRNPA1 ENST00000547276 or NM_002136	Group 1	Group 0	0.0009	Group 2	Group 0	0.0028
HNRNPA1 ENST00000547276 or NM_002136	Group 2	Group 0	0.0019	Group 1	Group 0	0.0005
KIF6 ENST00000373213 or NM_001289020	Group 3	Group 2		Group 3	Group 2	0.7470
KIF6 ENST00000373213 or NM_001289020	Group 4	Group 2		Group 4	Group 2	1.0000
KIF6 ENST00000373213 or NM_001289020	Group 4	Group 3		Group 4	Group 3	0.9612
KIF6 ENST00000373213 or NM_001289020	Group 4	Group 1	0.8435	Group 4	Group 1	0.1000
KIF6 ENST00000373213 or NM_001289020	Group 3	Group 1	0.6691	Group 3	Group 1	0.0883
KIF6 ENST00000373213 or NM_001289020	Group 2	Group 1	0.5692	Group 4	Group 0	0.0028
KIF6 ENST00000373213 or NM_001289020	Group 4	Group 0	0.1823	Group 2	Group 1	0.0120
KIF6 ENST00000373213 or NM_001289020	Group 1	Group 0	0.2298	Group 1	Group 0	0.0034
KIF6 ENST00000373213 or NM_001289020	Group 3	Group 0	0.0472	Group 3	Group 0	0.0004
KIF6 ENST00000373213 or NM_001289020	Group 2	Group 0	0.0210	Group 2	Group 0	<0.0001
LPIN1 ENST00000256720 or NM_145693	Group 2	Group 0	0.0028	Group 2	Group 0	0.0003
LPIN1 ENST00000256720 or NM_145693	Group 3	Group 0	0.0122	Group 3	Group 0	0.0008
LPIN1 ENST00000256720 or NM_145693	Group 2	Group 1	0.0567	Group 1	Group 0	0.0092
LPIN1 ENST00000256720 or NM_145693	Group 3	Group 1	0.0549	Group 2	Group 1	0.0634
LPIN1 ENST00000256720 or NM_145693	Group 1	Group 0	0.2850	Group 4	Group 0	0.0317
LPIN1 ENST00000256720 or NM_145693	Group 4	Group 0	0.1713	Group 3	Group 1	0.2592
LPIN1 ENST00000256720 or NM_145693	Group 4	Group 1	0.9958	Group 4	Group 1	1.0000
LPIN1 ENST00000256720 or NM_145693	Group 3	Group 2	1.0000	Group 3	Group 2	0.9399
LPIN1 ENST00000256720 or NM_145693	Group 4	Group 2	0.5420	Group 4	Group 3	0.3886
LPIN1 ENST00000256720 or NM_145693	Group 4	Group 3	0.3886	Group 4	Group 2	0.3018
MYLIP ENST00000349606	Group 3	Group 2	0.9923			
MYLIP ENST00000349606	Group 4	Group 2	1.0000			
MYLIP ENST00000349606	Group 4	Group 3	0.9991			
MYLIP ENST00000349606	Group 3	Group 1	0.9839			
MYLIP ENST00000349606	Group 4	Group 1	0.8887			
MYLIP ENST00000349606	Group 2	Group 1	0.7107			
MYLIP ENST00000349606	Group 4	Group 0	0.0041			
MYLIP ENST00000349606	Group 3	Group 0	0.0006			
MYLIP ENST00000349606	Group 1	Group 0	0.0006			
MYLIP ENST00000349606	Group 2	Group 0	0.0003			